

CLAIMS

1. A method of producing double low restorer lines of *Brassica napus* for Ogura cytoplasmic male sterility (cms) presenting radish introgression carrying the Rfo restorer gene deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from *Brassica oleracea*, and having a good agronomic value characterised by female fertility, a good transmission rate of Rfo and a high vegetative vigour, said method including the step of:
  - a) crossing double low cms lines of spring *Brassica napus* comprising a deleted radish insertion with the double low line of spring Drakkar for forming heterozygous restored plants of *Brassica napus*,
  - b) irradiating before meiosis the heterozygous restored plants obtained in step a) with gamma ray irradiation,
  - c) crossing pollen from flowers obtained in step b) with the cms double low spring Wesroona line,
  - d) testing the progeny for vigour, female fertility and transmission rate of the cms gene,
  - e) selecting progeny lines.
- 20 2. A method according to claim 1, wherein the irradiation dose in step b) is 65 Gray during 6 mn.
3. A method according to claim 1 wherein the double low cms line of spring *Brassica napus* of step a) is R211.
- 25 4. A method according to claim 1 wherein the testing in step d) is performed with the combination of five markers selected from PG101, PG1UNT, PG1Int, BolJon and CP418.
- 30 5. Double low restorer lines of *Brassica napus* for Ogura cytoplasmic male sterility (cms) presenting a Rfo insertion deleted of the radish Pgi-2 allele and recombined with the Pgi-2 gene from *Brassica oleracea*, and having a

good agronomic value characterised by female fertility, a good transmission rate of Rfo and a high vegetative vigour.

6. Double low restorer lines of Brassica napus according to claim 5, wherein  
5 they present a unique combination of five markers selected from PGIol,  
PGIUNT, PGIint, BolJon and CP418.
7. Brassica napus hybrid plants and progeny thereof obtained through the  
steps of:
  - 10 a) providing a restorer line produced according to claim 1 and bred to be homozygous,
  - b) using said restorer line in a hybrid production field as the pollinator,
  - c) using cms sterile plants in a hybrid production field as the hybrid seed producing plant, and
  - 15 d) harvesting the hybrid seed from the male sterile plant.
8. The seeds of Brassica plant developed from the Brassica line obtained in  
claim 1.
- 20 9. The seeds of Brassica napus obtained in claim 7.
10. The seeds of Brassica napus obtained in claims 1 and 2 deposited in NCIMB  
Limited, 23 St Machar Drive, Aberdeen, Scotland, AB24 3RY, UK, on  
July 4 , 2003, under the reference number NCIMB41183.  
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11. Use of the combination of at least four markers PGIol, PGIint, BolJon and  
CP418, or any portion of them comprising at least one polymorphic site, for  
characterising recombined restorer lines of Brassica napus for Ogura cms  
presenting a Rfo insertion deleted of the radish Pgi-2 allele and recombined  
30 with the Pgi-2 gene from Brassica oleracea, and having a good agronomic  
value characterised by female fertility, a good transmission rate of Rfo and a  
high vegetative vigour.

12. Use according to claim 11 wherein the combination is of five markers PGIol, PGIUNT, PGInt, BolJon and CP418.
- 5 13. Use according to claim 12, wherein:
- The marker PGIol is amplified using the primers: PGIol U and PGIol L  
(PGIol U: 5'TCATTTGATTGTTGCGCCTG3';  
PGIol L: 5'TGTACATCAGACCCGGTAGAAAAA3' )
  - The marker PGInt is amplified using the primers: PGInt U and PGInt L  
(PGInt U: 5'CAGCACTAATCTTGCCTGATG3';  
PGInt L: 5'CAATAACCCTAAAAGCACCTG3' )
  - The marker PGIUNT is amplified using the primers: PGIol U and PGInt L:  
(PGIol U: 5'TCATTTGATTGTTGCGCCTG3';  
PGInt L: 5'CAATAACCCTAAAAGCACCTG3' )
  - The marker BolJon is amplified using the primers: BolJon U and BolJon L:  
(BolJon U: 5'GATCCGATTCTTCTCCTGTTG3';  
BolJon L: 5'GCCTACTCCTCAAATCACTCT3' )
  - The marker CP418 is amplified using the primers: SG129 U and pCP418 L:  
(SG129 U: cf Giancola et al (5)  
20 pCP418 L : 5'AATTCTCCATCACAAAGGACC3' )

14. PGIol marker whose sequence follows:

25 TCATTTGATT GTTGCCTG TCGCCTTGT GTGTTATGAT GAATGAACAG CAGTCATT 60  
ACATGTGGTT AACTAACAG GGCTCCGGCT GTTGCAAAAC ACATGGTTGC TGTCAGCACT 120  
AATCTTGCCTG TATGAATTG TGATTAATT TGTTGTTG TGACTCTTC TTCATTGTT 180  
GTTTCTGTA AATAAACCGA ATGTATAATC TTTTACAAA CTGAATTTC TACCGGGTCT 240  
GATGTACA 248

15. PGIUNT marker whose sequence follows:

30 TCATTTGATT GTTGCCTG TCGCCTTGT GTGTTATGAT GAATGAACAG CAGTCATT 60  
ACATGTGGTT AACTAACAG GGCTCCGGCT GTTGCAAAAC ACATGGTTGC TGTCAGCACT 120  
AATCTTGCCTG TATGAATTG TGATTAATT TGTTGTTG TGACTCTTC TTCATTGTT 180  
GTTTCTGTA AATAAACCGA ATGTATAATC TTTTACAAA CTGAATTTC TACCGGGTCT 240  
ATGTACAATG CTAGTCTCCA TGTCTTGGG GATCATGATT 300  
35 ACAGTACAGA AGAAAAGTGT CAAAACCTG GATGTTTAA TTTACAGTTA GTGGAGAAGT 360  
TCGGCATTGA TCCGAACAAT GCATTTGCAT TTTGGGACTG GGTTGGTGA AGGTACAGTG 420  
GTAAGTGCTT GTTATTGG TTGTATAAT TTCTCGTCCA TTTCCGCTTG CTTAGTGTAT 480  
AACTGAAATT CTTTGCAGT TTGCAGTGCT GTGGAGTCT TACCATGTC TCTACAGTAT 540  
GGCTTCTCTG TGGTTGAGAA GTACGGTACC TTCTACTTTA TCAGCCATCT CATAAAATGT 600

5 CTTAGGCATA TTCTTCTAT TTTATTCCTC TCTTAATGAT TTCTTCTTT TTTTATTGCA 660  
 TCCCGTTT ATTTCAAAA GTGTTACTG TCTCTAAATC AAGAAGAAC CTTCTTAGTA 720  
 GATCCAGCTG ATATTCAAGCC TTTTTAAAT TGGACTGCAG GTTTTAAAG GGGAGCTTC 780  
 ACCATTGATA AGCATTTCCA GTCCACACCG TTTGAGAAGA ATATACCCGT GAGTTGCATT 840  
 AGTTGTGTGA TTATACAGTT TTCTTGTCTT TTTGCTATGT CCATCAACAC TAGAGATTG 900  
 TGAAGTTATT AGTGTAGTCA ACGCATAGGG AGAGGTGATT GGTGACTTT GGACGATTTC 960  
 AGGTGCTTTA GGGTTATTG 979

16. PGIint marker whose sequence follows:

10 CAGCACTAAT CTTGCGGTAT GAATTTGTGA TTAAATTGTT TTGTTTGTA CTCTTCTTC 60  
 ATTGTTCGTT TTCGTACAAT AAACCGAATG TATAATCTTT TACAAACTGA ATTTTCTACC 120  
 GGGTCTGATG TACAATGCTA GTCTCCATGT TCTTGGGGAT CATGATTAT TTTCTACATG 180  
 TATTCAAGACA GTACAGAAGA AAGTGTCAA AACTCTGGAT GTTTTAATT ACAGTTAGTG 240  
 GAGAAGTTCG GCATTGATCC GAACAAATGCA TTTGCATT TTGACTGGGT TGTTGGAAGG 300  
 15 TACAGTGGTA ACTGCTTGTG TATTGTTG TATAAATTTC TCGTCCATT CCGCTTGCTT 360  
 AGTGTATAAC TGAAATTCTT TTGCAGTTG CAGTGTGTT GGAGTCTAC CATTGTCCT 420  
 ACAGTATGGC TTCTCTGTGG TTGAGAAGTA CGGTACCTTC TACTTTATCA GCCATCTCAT 480  
 AAAATGTCTT AGGCATATTTC TTCTTATTG ATTTCCCTCT TAATGATTTC TTCTTTTTT 540  
 TATTGCAATT CCGTTTATT TTCAAAAGTT GTTACTGTCT CTAATCAAG AAGAAACCTT 600  
 20 CTAGTAGAT CCAGCTGATA TTCAGCCTT TTAAATTGG ACTGCAGGTT TTAAAGGGG 660  
 AGCTTCAAGC ATTGATAAGC ATTTCCAGTC CACACCGTTT GAGAAGATA TACCCGTGAG 720  
 TTGCATTAGT TGTGTGATTA TACAGTTTC TTGTCTTTT GCTATGTCCA TCAACACTAG 780  
 AGATTGCGTA AGTTATTAGT GTAGTCAACG CATAGGGAGA GGTGATTGGT GACTTTGGA 840  
 CGATTCAGG TGCTTAGGG TTATTG 866

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17. BolJon marker whose sequence follows:

30 GATCCGATTTC TTCTCCTGTT GAGATCAGCT CCAAACATCA AACAACTTGT ACACAAATAT 60  
 CTTTACTTGC TAAATGGAAC ATGACAAGAG ATAGAAAATC TTGCTCATAG TATTGTACAA 120  
 GGGATAACAG TGTAGAAAAC AAACCGTCTG TAAGATTTTC TCCCTGATCC TCTCACTTAA 180  
 CCAGTAGGCG TTTTCACAT TGAAGCGCAT ATCTACTTTG GTATTCACTG AATAAAAAAA 240  
 GAAAGCTGGT AACATGTGAA GGATATACAA GCATTGATAC ACCAAGTAGT CACAAACTAC 300  
 ATTATAAAGG TCAGACCTTT GTTCACATTC TGGCCTCCAG GACCACCGCT TCTAGCAAAG 360  
 TTAAGCGTAA CATGGTCTGC ACGTATACAA ATGAAAATGT TTCTATCAA ATCCTATAAA 420  
 ATAGAGCTCT ATAACATTGT CGATACATAG TTTCACTAAC TCTGCAGTA CTAACACAT 480  
 35 ATACAAACAA AACTATGCGA ACAGATCAAA ACTACTACAG AACACAGTTC TATGACACTG 540  
 TCGATAGTAA CATCCTCTGC AAGTACCAAA GAGATAGCAA ATGAAACTAT GTAAACAAAT 600  
 CAAAATTCTA AATTCTCCA TCACAAGGAC CTACAGAATA GAGTTATCAT AACATTTCCT 660  
 GTAAATATT CCATCAAAT GACTAGAGAA CAGAGTTCTT ATAACATTAT ATAACATTAT CTGTAATGT 720  
 40 TCCAACAAAA CCACTACATA GCAGAGTTCT TATAACATTG TCTGTAAATG TCCAATCAA 780  
 ACCACTACAG AACAAAGCTC CTATAACATT GTTATACAA AGTTCACTA AATCTACAAA 840  
 CTTTCCCCGT AAATGAGCTT AATATCACCC AAAGATGTTT CAATCAGATA AAATCAGATA AAGAGTACGA 900  
 CATCGTTTG AGATTAGAAC AAACTGAAAC TTACGTAGAG TGATTGAGG AGTAGG 957

18. CP418 marker whose sequence follows:

45 AATTCTCCA TCACAAGGAC CTACAGAATA GAGTTATCAT AACATTTCCT GTAAATATT 60  
 CCATCAAAAT GACTAGAGAA CAGAGTTCTT ATAACATTAT CTGTAATGT TCCAACAAAA 120  
 CCACTACATA GCAGAGTTCT TATAACATTG TCTGTAAATG TCCAATCAA ACCACTACAG 180  
 AACAAAGCTC CTATAACATT GTTATACAA AGTTCACTA AATCTACAAA CTTTCCCCGT 240  
 AAATGAGCTT AAATCACCC AAAGATGTTT CAATCAGATA AAGAGTAACG ACATCGTTT 300  
 50 GAGATTAGAA CAAACTGAAA CTTACGTAGA GTGATTGAG GAGTAGGCTC GTGCCAGCA 360  
 GAGCTAGCTC TCTCCTCCGC CTATGAAGC ATCTGTTGCA CCTGAGACAA CGTGACGAA 420  
 ACTTTCCGAT CACGCCACC AGAATTGAC GCGCGCATC GGAAGGATCC GAATCGGGAA 480  
 CTGAGTGAAC CCGAGCGATC CCGGGAGTGC GACGGAGCGA TGGGAAAAGA GAGTGGCAGC 540  
 ATTCGACGA AGAGTGGAAAG AGGAGAGGGT GGTGGATAAA CTCGCGTATG ATCAAGTTG 600  
 55 TCATCGTCCT GATTGCCGCC ATTTTTTG TCAGGGCGCT CTGTGGCTTA GAAGTTCCG 660  
 atgtcaatga ac 672